Floral abundance and resource quality influence pollinator choice

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Title: Floral abundance and resource quality influence pollinator choice

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Running Title: Floral rewards drive pollinator choice

Abstract

1. Pollinator declines caused by forage habitat loss threaten insect pollination services. Pollinating insects depend on adequate floral resources, and their ability to track these resources. Variability of these resources and the effect on insect foraging choice is poorly understood.
2. We record patterns of visitation to six wildflower species and test the hypotheses that: pollinators preferentially visit the most rewarding flowers; nectar diurnal variations affect foraging preferences; pollinators respond most strongly to nectar rewards.
3. Nectar volume and sugar concentration were negatively correlated within plant species over time of day where greater concentration and lower volume was evident in the afternoon, but this did not correspond to pollinator visitation. Both floral abundance and nectar quality (total sugar per inflorescence) positively affect insect visitation. For some foragers the positive effects of high quality rewards were only evident when floral abundance was high (>50 inflorescences per patch), perhaps reflecting the low probability of pollinators detecting scarce rewards. Pollen quality (total protein per inflorescence) was negatively related to visitation of *Apis mellifera* and *Bombus pascuorum*.
4. Fewer pollinators visiting flowers of higher pollen quality could reflect plant allocation trade-offs or the presence of secondary metabolites in pollen, meaning pollen foraging is likely affected by factors other than protein concentration. Nectar rather than pollen appeared to be the main driver of floral choice by insects in this system.
5. Conservation schemes for bees in farmland or gardens might benefit from ensuring that rewarding plant species are present at high density and/or are aggregated in space.

Introduction

Over the past 80 years, local and UK-wide changes in farming practice and agricultural intensification have led to a reduction in diversity of crop and non-crop plants (Robinson & Sutherland, 2002; Öckinger *et al.*, 2007; Geiger *et al.*, 2010). This includes the loss of meadow plants (Goulson *et al.*, 2005), arable weeds and hedgerows (Hanley & Wilkins, 2015), which provide valuable foraging resources for flower visiting insects. This habitat degradation has been identified as the primary reason for population declines in insects reliant on flowers to provide nectar and/or pollen as a main food source, including adult butterflies (Brereton *et al.*, 2011) and adult and offspring honey bees (Potts *et al.*, 2010), bumblebees (Goulson *et al.*, 2008) and solitary bees (Ollerton *et al.*, 2014). Nesting and hibernation resources aside, it is imperative that bees and other pollinating insects are able to forage effectively for nutritional resources in increasingly fragmented landscapes in order to survive and reproduce, particularly as they are facing other pressures such as diseases and pathogens (Cox-Foster *et al.*, 2007), global environmental change (Tylianakis *et al.*, 2008) and pesticide use (Goulson *et al.*, 2015), all of which impact on their survival. Population decline in flower visiting insects could jeopardise the pollination services provided to entomophilous crops (Kremen *et al.*, 2002; Klein *et al.*, 2007).

Nectar provides sugars (mainly sucrose, glucose and fructose), which energise pollinators to continue foraging (on nectar or other sources of nutrients), undertake nesting activities, find mates, and provide for offspring. It also contains ions, water and small amounts of amino acids, which may contribute to nutrition (Kim & Smith, 2000). Pollen comprises proteins, carbohydrates, lipids, vitamins and minerals (Roulston & Cane, 2000; Nicolson, 2011). Although many flower visiting insects consume pollen for sustenance (e.g. beetles, flies), bees (Hymenoptera: Apoidea) also collect pollen to feed larvae, and many simultaneously collect both nectar and pollen from flowers, depending on what requirements they have and what flower species they are feeding on (Heinrich, 1979a; Heinrich, 1979b; Goulson *et al*., 2005).

Survival and reproductive success of pollinating insects is dependent on them successfully gathering adequate protein and sugar to provide for their energy and nutritional needs. For example, many Lepidoptera, Diptera and Hymenoptera rely on energy gained from nectar to undertake mating flights, for dispersal and/or migration, and to find suitable places to lay their eggs. Butterflies tend to exhibit lower fecundity when nectar limited (Boggs & Ross, 1993), and the hoverfly *Episyrphus balteatus* has greater longevity when fed a sugar and protein rich diet (Pinheiro *et al*., 2015). Bees require both pollen and nectar to feed to their offspring. When fed on protein rich diets, bumblebee colonies are more reproductively successful (Génissel *et al.*, 2002; Kitaoka & Nieh, 2009), and the solitary bee *Lasioglossum zephyrum* (Roulston & Cane, 2002) and honey bees (Basualdo *et al.*, 2012) produce larger adults. Larger bees generate and retain heat faster leading to earlier and more frequent forage flights (Stone, 1993), they have greater diapause survival (Strohm and Linsenmair, 1997) and are better able to cope with adverse conditions such as parasitism and disease (Alaux *et al.*, 2010; Di Pasquale *et al.*, 2013).

In order to forage effectively, pollinators use olfactory cues to enable detection of non-depleted nectar resources (Goulson *et al.*, 1998a; Howell & Alarcón, 2007) and greater nectar volume and sugar content (Pyke, 1978; Heinrich, 1979a; Wolff, 2006). Likewise, insect foragers tend to select pollen with greater protein and essential amino acid content (Levin & Bohart, 1955; Schmidt, 1982; Robertson *et al.*, 1999; Cook *et al.*, 2003; Arenas & Farina, 2012), and obligate insect pollinated plant species produce pollen that is richer in protein and amino acids compared to facultative species (Hanley *et al.*, 2008, but see Roulston *et al*., 2000).

Few studies have investigated how eusocial or solitary bees integrate information on nectar and pollen quantity and quality simultaneously, and those that have, indicate that nectar is the primary factor influencing foraging preference for bees, and pollen a secondary consideration (Konzmann & Lunau, 2014; but see Somme *et al.*, 2015). In addition, studies focusing on flower selection by pollinators tend to look only at individual species of bee and specific pollinators for individual plant species (e.g. Robertson *et al.*, 1999), or solely bees as a group (e.g. Heinrich, 1979b; Pernal & Currie, 2001). Floral choice tests are frequently undertaken in controlled conditions (e.g. Konzmann & Lunau, 2014), but the limited number of field studies investigating the influence of rewards on the foraging decision of pollinators makes it difficult for the conclusions gained by laboratory studies to be applied, especially as there are external factors in the natural environment likely to influence the results, such as the spatial distribution and reward phenology of flowers. For example, Hanley & Wilkins (2015) describe greater food plant abundance in roadside, compared with field facing hedgerows, and noted a corresponding increase in bumblebee abundance. In addition, several studies indicate that some flower species offer less nectar in the middle of the day and afternoon compared to the morning and evening (Mačukanović-Jocić *et al.*, 2004; Silva *et al.*, 2004; Mačukanović-Jocić & Đurđević, 2005), providing a further challenge to efficient foraging by pollinators since the relative rewards provided by different flower species may alter hour by hour through the day.

In this study, we examine the foraging choices made by all flower visiting insects in relation to the relative nectar and pollen quantity and quality of six common plant species under natural conditions. We tested the hypothesis that nectar quantity or quality shows diurnal variation and that nectar or pollen quantity or quality, or a combination of reward metrics, predicts insect visits. We record how nectar volume and sugar concentration changes through the day, and also the pollen weight and protein concentration for each test plant species, and relate these values to insect flower choices through the day. Specifically, this study's objectives were to; (i) record nectar and pollen quantity and quality estimates for six test plant species, (ii) investigate the diurnal variation in nectar quantity and quality for the test plant species, and (iii) assess how nectar and pollen quantity and quality influence foraging choices by flower visiting insects.

Methods

Test Plant Species

Six species of flowering plant that are common in southern UK were selected for nectar and pollen quantity and quality estimation, and insect visitation surveys in the field. These were *Lamium album* L. and *Glechoma hederacea* L. (Lamiaceae), *Crataegus monogyna* Jacq. and *Rubus fruticosus* L. agg. (Rosaceae), Symphytum *officinale* L. (Boraginaceae) and *Ranunculus repens* L. (Ranunculaceae). These spring or early summer flowering plants considered to be beneficial foraging plants for insects in previous studies (Lack, 1982; Goulson *et al.*, 1998b; Kipling & Warren, 2013) (further species details are listed in Table S1 in the Electronic Supplementary Material). Previously, *R. fruticosus* and *S. officinale* were included in studies of pollen protein quality and its effects on bee foraging preferences (Hanley *et al.*, 2008), but to our knowledge the other test plant species have not been studied in this context.

Site Selection

Sites were chosen to be included in this study if they contained at least three of the test plant species within 50 m of each other, were on chalk soil, were easy to access and were subject to intermediate levels of disturbance (e.g. mowed once a year, or grazed intermittently by sheep). Sites could be divided into two groups, road verges and semi-improved grassland. In terms of management, road verge sites were mowed once a year and semi-improved grassland were grazed several times throughout the year. However, none of the survey sites were either mown or grazed whilst this study was taking place. Once sites were identified, nectar and pollen samples were collected from test plant species and visitation sampling was conducted.

Nectar Volume and Sugar Concentration

Nectar sampling was undertaken on 30 inflorescences of each test plant species, collected from at least three different survey sites. Nectar was sampled from inflorescences of plant species that had recently come in to bloom and were easily accessible. For each inflorescence nectar production was estimated from morning, afternoon and evening periods for each plant species tested. For each species then flowers were emptied of nectar and bagged by 09:00 (GMT) using a fine-mesh cotton fabric and masking tape. At 15:00, 21:00 and 09:00 the following morning flowers were emptied of nectar using 5 µl micropipettes (BLAUBRAND® intraMARK, Wertheim, Germany). The nectar from each inflorescence was then expelled onto a field refractometer (Bellingham and Stanley Ltd, Basingstoke, Hants, UK) to measure the proportion of sugar in each sample (Bolten *et al.*, 1979). This produced both mean nectar volume and mean sugar concentration for each species in each time period, and over a 24 hour period when totalled.

Pollen Weight and Protein Concentration

Pollen was collected from 10 inflorescences from at least three different sites for each plant species. Standing crop of pollen was taken just once for each replicate so variation in pollen quantity or quality was not measured over time of day. Flowers were collected in the field before anthers had dehisced, and placed in water in an unheated and well ventilated laboratory until anthers opened. Pollen was collected systematically until anthers were empty. It is conceivable that placing flowers in water affects pollen quality, but all plant species were treated in the same way. Pollen was either stored in a – 20 °C freezer for drying at a later date, or immediately dried in an oven for 24 hours at 40 °C. After drying, pollen was weighed to measure total pollen weight per inflorescence. Protein extraction and detection was undertaken using the Bradford assay (Bradford, 1976). The assay binds protein to Coomassie Brilliant Blue G-250 dye (4.7% [weight: volume] ethanol, and 8.5% [weight: volume] phosphoric acid dissolved in water). Light absorbance is then measured against known protein standards. From each of the 10 inflorescence pollen from each species, 1 mg pollen were dusted with aluminium powder, wetted with 20 µl 0.1 mol/L NaOH and ground with a micro-pestle. Ground pollen was reanimated with 480 µl 0.1 mol/L NaOH and placed in a refrigerator for 24 hours before analysis, but used within 1 week. Prior to absorbance measurement samples were placed in boiling water for 5 minutes and centrifuged for 5 minutes. Then 10 µl of supernatant was slowly vortexed with 300 µl of dye reagent. This was repeated in triplicate for each sample and left to incubate at room temperature for 15 minutes.

Protein standards were made up each time samples were run, using pre-mixed concentrations of Bovine Serum Albimum (BSA) from the BIO-RAD (Hertfordshire, UK) Quick-Start™ Bradford Protein Assay kit. Once samples and standards were created, they were measured for absorbance within an hour of mixing at 595 nm using a Thermo Scientific (Paisley, UK) Nanodrop 2000 UV-Vis Spectrophotometer. This produced the standing crop of both mean pollen weight and a crude mean protein concentration for each test plant species.

Visitation Surveys

Visitation surveys took place at seven sites across Sussex, UK between May and June 2014 (for site details see Table S2). Surveys were undertaken in the morning (08:00—10:00), afternoon (13:00—15:00) and evening (19:00—21:00). Each survey consisted of a 10 minute standing observation of a 4 m^2 area of each test plant species at each site. The number and species of all visiting insects to that plant species were recorded. All observed flower visiting insects appeared to collect nectar and, in most cases, pollen from plant species, therefore we did not attempt to distinguish which resources insects were collecting. The number of inflorescences of the plant species, ambient temperature, and wind speed were recorded for each survey. Surveys were only conducted when air temperature was > 14°C and average wind speeds was < 20 mph.

Common species of bumblebee (genus *Bombus*) and European honeybees (*Apis mellifera* L.) were identified on the wing. Due to the unreliability of morphological characters for separating *Bombus terrestris* L. and *B. lucorum* agg. workers in the field, these two species were grouped. Although others have recorded flower visiting bumblebees into groups based on colour type (Haughton *et al*., 2003), in this study the bumblebee species observed were easily separated to species in the field. However, faded or suspected cleptoparasitic bees (*Psithyrus* spp.) were caught and checked using a hand magnifying glass. Other visiting insects too small to be identified on the wing were collected and identified to species or genus. Although unlikely given the short period of each survey, the possibility of double counting insects was the same for all surveys due to equal sampling time and therefore assumed to be unbiased across the study.

Statistical Analysis

Means tests of variance were used to assess differences in floral resources available from the plant species, as well as the number of insect visits, between periods of the day. Based on whether resource and insect visit metrics showed homogeneity of variance between species or periods of the day using Levene's test, either ANOVA (parametric) or Kruskal-Wallis (non-parametric) tests were applied to assess variance for all groups before either Tukey HSD tests or pairwise Wilcox tests were applied to assess differences between groups, respectively.

Generalized Linear Mixed Models (GLMMs) were used to test the effects of resource metrics on insect visitation (i.e. counts per 10 minute survey). Prior to applying models, proposed explanatory variables were checked for multicollinearity using Variance Inflation Factors (VIFs), if variables had VIFs greater than 3 or correlation coefficients more than 0.6 with other variables, they were excluded from models (Zuur *et al.*, 2010). These analyses indicated nectar volume and pollen weight were correlated with sugar and protein concentration, respectively. To enable modelling of resource quality and quantity without violating model assumptions, nectar volume and pollen weight were multiplied by sugar and protein concentration to create total sugar and protein in milligrams per inflorescence as measurements of quality in the plant species. To account for the change in density of the solution based on the amount of sugar recorded, percentage sugar was multiplied by the mass density (g/cm3) of sugar at that concentration. These measurements also allowed the interpretation of the nutritional gain available to foraging insect pollinators, and is referred to as total sugar and total protein from here on.

GLMMs were initially run with the environmental variables temperature and wind speed included as explanatory variables, however these had no effect on visitation or the outcome of the models so were removed.

We modelled five visitation response variables (including: all insects; all bumblebees; the three most recorded species, *B. pascuorum* Scopoli, *B. pratorum* L., and *A. mellifera*) in response to the number of inflorescences (log transformed) for each test plant species surveyed in each observation area (4 m^2) (floral abundance), and total sugar and protein. We did not analyse data on other groups of flower visiting insects alone as they were recorded in such small numbers that data analysis would have been unreliable. However, they were included in the data analysis as part of ‘all insects’ in statistical models. We included interactions between floral abundance and total sugar and protein explanatory variables. Sample site was fitted as a random factor because observations were nested within sample sites and contained different combinations of test plant species. Visitation rates were count data, therefore models were applied with Poisson errors (O’Hara & Kotze, 2010). Model residuals were assessed for normality and heteroscedasticity. Model simplification was carried out using likelihood ratio tests, and sequentially deleting terms that did not significantly decrease model deviance, beginning with higher order interactions.

To further investigate how particular plant species affect insect visitation, linear mixed effects (LME) models were used to test the effects of floral abundance and species on the same response variables as the GLMMs, with site as a random factor. Models were tested for significance using likelihood ratio tests, with and without species as an explanatory variable.

Already published protein estimates for each plant species were compared to confirm the extraction results and accuracy of our values. We found that only *Lamium album* (4.48%) differed from the literature for *Lamium* sp. (22.8% in Roulston *et al*., 2000), so to assess whether this estimate affected our findings, we substituted it in the two models that included total protein (for *A. mellifera* and *B. pascuorum* visitation).

Statistical analysis was conducted using R v3.1.2 (R Core Team, 2014) within RStudio (RStudio, 2012) using packages ‘lme4’ (Bates *et al*., 2015) and ‘usdm’ (Naimi, 2013), and plots were generated using ‘ggplot2’ (Wickham, 2009).

Results

Nectar and Pollen Quantity and Quality

A large amount of between-inflorescence variation was found in nectar and pollen resources for each test plant species (Table 1). Nevertheless, nectar volume (Kruskal-Wallis H = 211.64, d.f. = 5, P < 0.001), nectar sugar concentration (H = 112.61, d.f. = 5, P < 0.001) and pollen protein concentration (ANOVA $F_{5, 54} = 20.6$, P < 0.001) varied significantly between plant species, with only pollen weight ($F_{5, 54} = 1.25$, P = 0.3) showing no significant differences.

Over 24 hours, *Rubus fruticosus* produced the greatest volume of nectar, which contained the lowest sugar concentration (Table 1). In contrast, *Ranunculus repens* produced the least nectar, whilst *Crataegus monogyna* offered the highest sugar concentration. *Lamium album* pollen offered the greatest mass per inflorescence but had the lowest protein concentration of all species. *C. monogyna* produced the least pollen mass whilst *G. hederacea* offered the highest protein concentration. We found no correlation between nectar and pollen quantity (Pearson R = -0.46, P = 0.26) or quality (Pearson R = -0.33, P = 0.52).

Nectar volume and pollen weight were multiplied by sugar and protein concentration, respectively, to create total sugar and protein in milligrams per inflorescence (Table 1). Over 24 hours, *Symphytum officinale* produced the greatest total sugar per inflorescence whilst *R. repens* produced the least. *C. monogyna* contained the most total protein per inflorescence and *S. officinale* the least (Table 1).

Diurnal Variation in Nectar Quantity and Quality

Mean nectar volume and sugar concentration averaged between plant species (regardless of time period) were not significantly correlated (Pearson R = -0.49, P = 0.32, Fig. 1A). When mean for each species at each time period was taken, there was a significant negative correlation between nectar volume and sugar concentration (Pearson R = -0.52, P = 0.02, Fig. 1B).

Volumes of nectar produced differed markedly between species, and between morning, afternoon and evening periods. *R. fruticosus* produced the greatest volume of nectar of all species in the evening and morning, yet recorded the lowest sugar concentration of all species in these periods (Table 1). The lowest volume of nectar was detected in *R. repens* in the evening, and the greatest sugar concentration was found in *C. monogyna* in the afternoon. When averaged across species, mean nectar volume did not differ significantly between periods of the day ($F_{2, 15} = 1.18$, $P = 0.35$). However, mean nectar sugar concentration across species increased significantly in the afternoon compared to the morning ($F_{2, 15} = 4.01$, $P = 0.04$) (Fig. 2).

Once sugar and protein concentrations were calculated between time periods, total sugar was significantly lower in the afternoon period compared to the morning or evening for *R. fruticosus*, *G. hederacea, L. album* and *R. repens* (Fig. 3). The exceptions were *C. monogyna* which produced comparable amounts of total sugar in each time period sampled, and *S. officinale* which had marginally more total sugar in the afternoon compared to morning and evening periods.

Foraging choices of flower visiting insects

In total, we made 112 ten-minute observations (38, 39 and 35 in morning, afternoon and evening periods, respectively), between May and June 2014 at seven sites in Sussex, during which 574 insects were recorded visiting test plant species. Proportionately, of all insect visits, 93% were by bees (Hymenoptera: Apidae), 6% by Diptera (< 1% of which were hoverflies [Syrphidae]), < 1% by Lepidoptera and < 1% by Vespidae (Table S3). Of all flower visiting insects, the three most numerous were the bee species *Bombus pascuorum*, *B. pratorum,* and *Apis mellifera,* which represented 33%, 30% and 16% of all insect visits, respectively (Table S3). The majority of insects were recorded visiting *R. fruticosus* (47%), *S. officinale* (24%) and *L. album* (19%) (Table S3). LME models showed significant species-specific preferences for the most commonly recorded flower visiting insects (Table S4). *B. pascuorum* mainly visited *L. album* and *S. officinale* (49% and 31% of this species' visits, respectively), *B. pratorum* preferred *R. fruticosus* and *S. officinale* (57% and 36%), and *A. mellifera* mainly visited *R. fruticosus* (87%) (Fig. S1 & Table S3).

Fewer insects were recorded in the evening (122) than the morning (221) and afternoon periods (231), and differences in abundance were also evident within and between recorded insect and test plant species (Table S3). However, these differences were not significant when tested between surveys for all insect abundance ($F_{2, 109} = 2.59$, $P = 0.07$). No significant difference was found between any response variable between different periods of the day when all test plant species were grouped or tested separately, except *G. hederacea* which had significantly more insects visiting during the afternoon compared to morning and evening periods ($F_{2, 24} = 9.03$, P

= 0.001; Fig. 4). This however does not correspond with the timing of maximum sugar availability, which appeared highest in the morning or evening for most species (Fig. 3). As standing crop of pollen was taken just once for each replicate, we could not quantify variation in pollen quantity or quality over the day.

Overall, flower abundance had a positive effect on the total number of bumblebee (bees within the genera *Bombus*) and all insect visitation (Table 2). Total sugar predicted insect visitation, with this relationship being positive for all response categories apart from *B. pascuorum* which was negative (Table 2). There were also a number of significant higher order interactions between floral abundance and total sugar and protein. For *Apis mellifera*, all bumblebees and all insects, the relationship between insect visitation and total sugar was weak or absent at low floral abundance (10 – 50 inflorescences per 4 m^2) but positive at high floral abundance (51 – 1000 inflorescences per 4 m^2) (Fig. 5). Total protein had a significant negative effect on *A. mellifera* visitation (Table 2). In addition, a negative interaction between total protein and floral abundance was found for *B. pascuorum* (Table 2), with the relationship weak or absent at low floral abundance (10 – 50 inflorescences per 4 m^2) but negative at high floral abundance (51 – 1000 inflorescences per 4 m^2). However, the three plant species most visited by insects (*R. fruticosus*, *S. officinale* and *L. album*) were also recorded as producing the lowest total pollen protein. When the protein value for *L. album* was replaced with the higher value reported in Roulston *et al*. (2000) there was no longer a negative significant effect of total protein on *B. pascuorum* visitation, and we found a positive significant effect of total sugar (Table S5).

Discussion

Many insects forage on flowering plants to gain key nutritional resources, largely nectar and pollen. What factors determine when plants secrete nectar is still largely unknown, as both internal and external factors can affect the rate of secretion (Heil, 2011). In our study, floral rewards of nectar and pollen (apart from pollen weight) significantly differed between test plant species. Similarly, nectar resources varied between periods of the day, whilst the single measure of pollen for each replicate meant this resource could not be tested for diurnal differences. Nectar volume and sugar concentration were negatively correlated in test plant species, with sugar concentration greater in the afternoon, which corresponds with previous studies showing similar trends (Mačukanović-Jocić *et al*., 2004; Silva *et al*., 2004; Mačukanović-Jocić & Đurđević, 2005). This may be due to decreased water availability or flowers reabsorbing nectar in the afternoon (Silva *et al.*, 2004) when humidity decreases and temperatures rise (Silva *et al.*, 2004; Mačukanović-Jocić & Đurđević, 2005). While our results suggests that nectar resources vary in quantity and quality across the day, insect visitation did not track nectar availability (with the exception of *G. hederacea* where the opposite was found: more insects were recorded visiting this species in the afternoon when lower total sugar was recorded). This may be an indication that some pollinators are not capable of accurately responding to changes in nectar production throughout the day, or it may be that their activity is constrained by other factors such as temperature. It is important to note that our methods meant that afternoon and evening nectar sampling occurred at 6-hour intervals, compared with morning sampling which was undertaken 12 hours after the flowers had been emptied and bagged the previous evening. We considered this as providing a fair estimate of how much nectar is likely available to foraging insects by the morning, however, the effect of early morning foraging between 05:00 and 09:00 GMT was not represented by our data and this should be taken into account in interpretations.

Due to the high energy cost of foraging, successfully selecting the most rewarding flowers is predicted to have a large impact on survival and reproductive success, especially when floral resources are fragmented. The most documented resource offered to insects by plants as an attractant for pollination is nectar. Foraging insects are capable of learning nectar rewards gained from visited flowers (Pyke, 1978), preferring to forage on flowers with significantly more nectar (Heinrich, 1979a; Wolff, 2006) and with greater sugar concentration (Hendriksma *et al.*, 2014). After testing for relationships between nectar and pollen resources and pollinator visitations our results support this, as typically, we found that greater nectar resources had a positive effect on insect and bee visitation. Single species specialism in insect-plant mutualisms is rare (Waser *et al.*, 1996), and the majority of flower visiting insects have flexible foraging choices. Foraging bumblebees show flower constancy (‘majoring’ on one particular species of known reward) and flower infidelity (‘minoring’ on other flowers checking reward change over time) (Heinrich, 1979b). This behaviour allows foragers to track resources in multiple flower species in a habitat. In our study, the positive effects of high floral rewards i.e. sugar were often only apparent when the floral abundance of test plant species was high (> 50 inflorescences), with scarce flowers tending to be visited less frequently even when comparatively highly rewarding. Our results appear to support Heinrich (1979b); if a flower species is both abundant and rewarding then insects are very likely to have discovered its value and preferentially visit it. In addition, where there are more flowers in a patch, there is a greater total quantity of nectar. Hence, although quality has an influence on forager choice, the abundance of floral rewards in the local environment is important in insect selection of resources.

There is evidence that bees show preferences for pollen with higher protein (Levin & Bohart, 1955; Robertson *et al.*, 1999) and essential amino acid content (Cook *et al.*, 2003), and these preferences are supported by obligate insect pollinated plant species producing pollen that is richer in protein and amino acids (Hanley *et al.*, 2008). However, other studies describe contrasting results where protein-rich pollen seems to be preferred in some cases, but not in others (Wille *et al.*, 1985 in Praz *et al.*, 2008; Roulston & Cane, 2002). Our results suggest greater total protein was negatively related to visitation by *A. mellifera*, and for *B. pascuorum* the negative relationship between protein content and visitation was more apparent when the floral abundance of test plant species was high (> 50 inflorescences). There are several possible explanations. Firstly, although not significant, there was a negative relationship between nectar and pollen quantity per inflorescence (correlation coefficient -0.46), so if bees are basing decisions primarily on nectar rewards they will tend to visit flowers with less pollen. We did not attempt to discern whether bees were collecting pollen only, nectar only, or both, but previous studies suggest that the majority of visits are for nectar (e.g. Goulson *et al*., 2005). Second, bees may have been responding to other nutritional compounds present in pollen. Plant species may be making trade-offs between protein and other nutritional elements that drive foraging preferences such as sterols (Somme *et al.*, 2014), lipids and starch (Roulston & Cane, 2000) or pollen-specific odours (Dötterl & Vereecken, 2010) not addressed in this study. Third, some plant species protect their pollen with defensive secondary compounds that may affect bee foraging choices (Gosselin *et al.*, 2013). For example, *Echium vulgare* has high protein content and also high concentrations of the hepatotoxins 1,2-dehydropyrrolizidine alkaloids and their N-oxides (Boppré *et al.*, 2005), which can be toxic to insects (e.g. Boppré *et al.*, 2005; Sedivy *et al.*, 2012) and affect flower selection (Kessler & Halitschke, 2009). In this study the species recorded with the greatest amount of pollen protein were *Glechoma hederacea* and *Ranunculus repens*, and pollen of *Ranunculus* spp. is known to contain the toxic lactone protoanemonin ranunculin with reportedly negative effects on honeybees (Sedivy *et al.*, 2012; Jurgens & Dotterl, 2004); and *G. hederacea* is toxic to some species of herbivorous insects as it produces a defensive insecticide protein (Hutchings & Price, 1999; Van Damme, 2008). It is also important to note that the negative effect we found did not persist for *B. pascuorum* once our values for *Lamium album* were replaced with higher values found in the literature. Our methods for extracting and measuring protein content are crude, and results can be variable so should be treated with caution. More detailed investigations are needed in which the full range of compounds present in pollen are quantified if we are to fully understand how bees choose which flowers to visit when collecting pollen.

Most studies do not measure both nectar and pollen rewards in relation to insect visit frequency, and in studies that do, conflicting results have been found. Konzmann & Lunau (2014) found that, in bumblebees, nectar rewards appear more important than pollen quality, whereas Somme *et al.*, (2014) found when pollen loads are analysed in conjunction with nectar from forage plants, both nectar and pollen quality appear important. In this study, while pollen and nectar are not negatively correlated, total nectar production appears to influence the visitation of insects to a greater extent than pollen. This could mean that flower visiting insects are more concerned with the quality of nectar, with pollen as a secondary consideration.

Insect visitation to test plant species appears to be species-specific, which can go further to explain our results. *B. pascuorum* mainly visited *L. album* and *S. officinale*, most likely due to its longer tongue allowing access to their deeper corollas. Contrary to other visiting species, we detected a negative effect of total sugar on *B. pascuorum* visitation. This may be because *B. pascuorum* had little competition from other insect foragers for *L. album* (only 16 other individuals recorded foraging on this species, which produced comparatively low amounts of sugar), or because this species was at the start of its life cycle when this study's sampling was undertaken and newly emerged queens were focusing foraging efforts on pollen collection. *Bombus pratorum,* which as a short-tongued bee may have exhibited restricted foraging choices, tending to visit flowers with greater total sugar. Although this species has a short tongue, it is a secondary nectar robber; 93% of recorded visits to *S. officinale* were via robbing (behaviour previously reported in Goulson *et al.*, 1998b). *Apis mellifera,* although also a short tongued species, mainly visited *R. fruticosus* and was not recorded robbing in this study, though it has been recorded acting as a secondary robber elsewhere (Darwin, 1872). Differences between foraging behaviour of bumblebees and honeybees suggest bumblebees (*B. terrestris* and *B. pascuorum*) show less fidelity when collecting pollen than honeybees, which have a highly flower-constant strategy (Leonhardt & Blüthgen, 2012).

Promoting and developing resources for pollinating insects is predominantly conducted through agri-environmental schemes promoting flower-rich field edges (Carvell *et al.*, 2007), or through targeted planting in urban spaces or private gardens (Hanley *et al.*, 2014). However, our understanding of the way in which pollinating insects respond to differences in the quality of resources offered by managed planting is limited. Our results suggest more consideration should be given when selecting plants for conservation management efforts, notably in terms of differing insect species requirements for pollen and nectar quality. Differences in flower selection between pollinator species may relate to the variation of life histories and may reduce competition for resources. Nectar resource quality appears to be the main driver of flower selection by most insect foragers in this study but, importantly, the benefits of greater resource quality in plants is dependent on local floral abundance. One practical conclusion to be drawn from this is that bees may benefit more from plantings of flowers (be they in farmland, parks or gardens) where species are presented in large clumps rather than in heterogeneous mixtures. More broadly, it is clear that there is still much that we do not understand about the role of sugars, proteins and other compounds in nectar and pollen in determining the foraging preferences of pollinators under field conditions.

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Table 1 Measurements per inflorescence of nectar (30 replicates) and pollen (10 replicates) quality and quantity (Mean ± SD) for *Crataegus monogyna*, *Rubus fruticosus*, *Glechoma hederacea*, *Lamium album*, *Ranunculus repens* and *Symphytum officinale* between times and totalled over 24 hours from seven sites in Sussex, UK.

Test Plant Species	**Nectar (µl)**				**Nectar (%)**				**Pollen (mg)**	**Pollen (%)**	**Sugar per Infl. (mg)**				**Protein per Infl. (mg)**
	Morning	Afternoon	Evening	**Over 24 Hours**	Morning	Afternoon	Evening	**Over 24 Hours**			Morning	Afternoon	Evening	**Over 24 Hours**	
Crataegus monogyna	1.1 ± 1.7	0.4 ± 0.4	0.4 ± 0.5	**1.9 ± 2.0**	26.13 ± 2.26	54.95 ± 3.15	36.54 ± 1.81	**35.03 ± 9.42**	**0.11 ± 0.03**	**15.39 ± 2.24**	0.6 ± 0.9	0.6 ± 0.1	0.3 ± 0.2	**1.2 ± 1.2**	**0.02 ± 0.01**
Rubus fruticosus	2.9 ± 2.8	0.9 ± 1.2	3.1 ± 2.1	**6.8 ± 3.7**	9.48 ± 0.52	24.41 ± 1.87	18.16 ± 1.26	**17.13 ± 4.72**	**0.48 ± 0.23**	**12.6 ± 2.28**	0.3 ± 0.2	0.3 ± 0.3	0.6 ± 0.5	**1.3 ± 0.7**	**0.06 ± 0.02**
Glechoma hederacea	1.2 ± 0.6	0.7 ± 0.4	0.8 ± 0.4	**2.6 ± 0.8**	29.03 ± 1.20	30.40 ± 1.57	29.43 ± 1.43	**29.86 ± 4.56**	**0.89 ± 0.29**	**19.07 ± 3.46**	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	**0.9 ± 0.2**	**0.17 ± 0.06**
Lamium album	1.1 ± 0.4	0.3 ± 0.2	0.8 ± 0.5	**2.2 ± 0.6**	19.76 ± 0.98	32.88 ± 2.13	27.67 ± 1.38	**25.88 ± 7.61**	**1.22 ± 0.33**	**4.48 ± 1.28**[1]	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	**0.6 ± 0.3**	**0.06 ± 0.02**
Ranunculus repens	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	**0.2 ± 0.2**	22.79 ± 2.25	28.00 ± NA	29.5 ± 2.60	**24.50 ± 8.13**	**0.76 ± 0.3**	**12.06 ± 1.50**	0.0 ± 0.0	0.1 ± NA	0.0 ± 0.0	**0.1 ± 0.0**	**0.09 ± 0.04**
Symphytum officinale	1.8 ± 0.8	1.3 ± 0.5	1.2 ± 0.6	**4.3 ± 1.1**	24.07 ± 1.06	44.59 ± 0.77	33.25 ± 1.61	**33.88 ± 4.60**	**0.95 ± 0.31**	**9.71 ± 1.38**	0.5 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	**1.7 ± 0.4**	**0.09 ± 0.03**

[1]This value differed from the previously recorded value of 22.8% in Roulston *et al*. (2000).
Bold values indicate the total for nectar measurements over 24 hours, and the total for pollen measurements (for which there was only a single measure per replicate)

Table 2 Generalized Linear Mixed Models (GLMM) test results investigating the effects of floral abundance, total nectar and pollen quality and interactions between floral abundance and each quality metric on visitation of insect foragers from seven sites in Sussex, UK. Displayed are parameter estimates ± standard error**,** z-value and p-value for each explanatory variable in the final model after simplification, and model AIC and log-likelihood tests. P-values are in bold if < 0.05 significance.

Visitation	**Explanatory Variables left in model**	**Parameter Estimate ± SE**	**z-value**	**p-value**	**AIC**	**logLik**
Apis mellifera	Log flower abundance	-0.066 ± 0.253	-0.261	0.794	331.2	-159.6
	Total sugar	7.845 ± 3.288	2.386	**0.017**		
	Total protein	-2.491 ± 0.927	-2.687	**0.007**		
	Log flower abundance × Total sugar	-1.405 ± 0.639	-2.201	**0.028**		
Bombus pratorum	Total sugar	2.838 ± 0.424	6.687	**< 0.001**	423.2	-208.6
Bombus pascuorum	Log flower abundance	0.281 ± 0.249	1.125	0.260	419.5	-203.7
	Total sugar	-1.380 ± 0.453	-3.048	**0.002**		
	Total protein	-12.584 ± 4.158	-2.785	**0.005**		
	Log flower abundance × Total protein	2.879 ± 0.888	3.241	**0.001**		
All *Bombus* Abundance	Log flower abundance	0.849 ± 0.168	5.049	**< 0.001**	605.5	-297.7
	Total sugar	6.275 ± 1.889	3.322	**< 0.001**		
	Log flower abundance × Total sugar	-1.074 ± 0.366	-2.932	**0.003**		
All Insect Abundance	Log flower abundance	0.425 ± 0.124	3.404	**< 0.001**	705.7	-347.9
	Total sugar	4.741 ± 1.522	3.113	**0.001**		
	Log flower abundance × Total sugar	-0.776 ± 0.296	-2.623	**0.008**		

Figures Captions

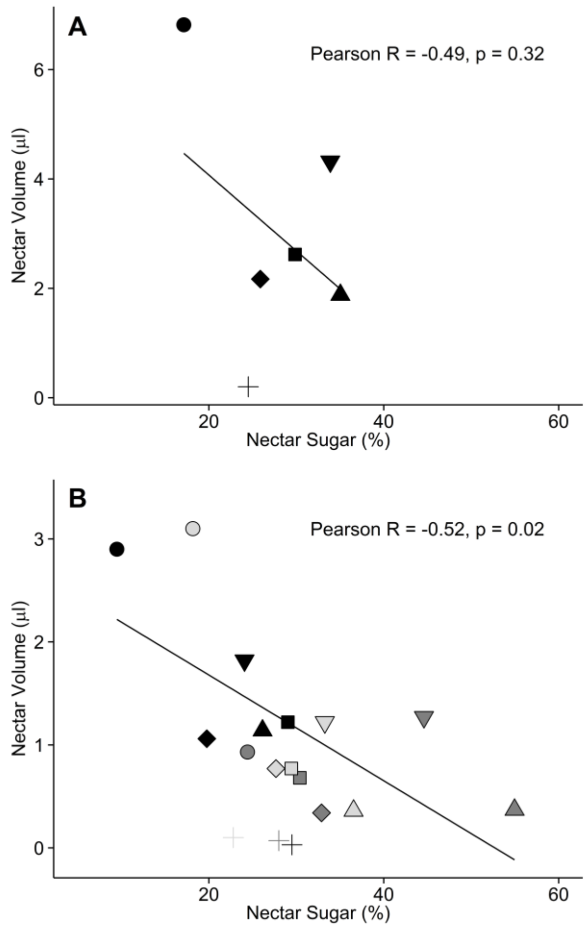

Fig. 1 Negative correlation between nectar volume (µl) and nectar sugar concentration (%) averaged between a) test plant species (Pearson R = -0.49, p = 0.32) and b) test plant species and time period (Pearson R = -0.52, p = 0.02). ▼ = *Symphytum officinale*, ▲ = *Crataegus monogyna*, ■ = *Glechoma hederacea*, ♦ = *Lamium album*, ● = *Rubus fruticosus* and + = *Ranunculus repens*. Black symbols = Morning (09:00 GMT), dark grey symbols = Afternoon (15:00) and light grey symbols = Evening (21:00) periods of nectar sampling.

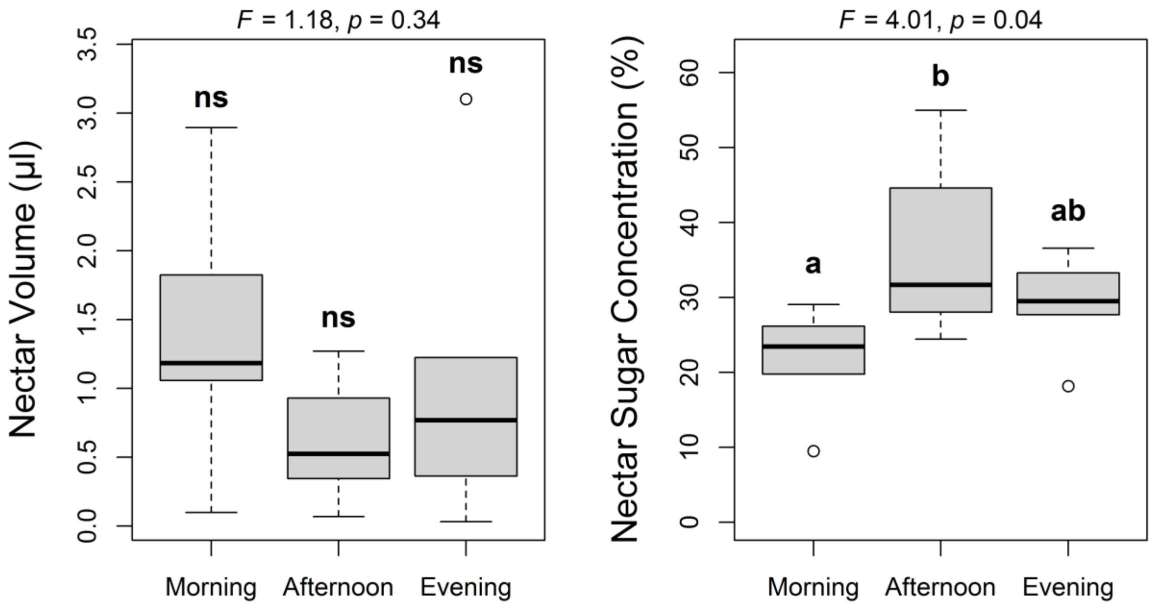

Fig. 2 The mean nectar volume (µl) and mean nectar sugar concentration (%) measured from 30 inflorescences in Morning (09:00 GMT), Afternoon (15:00) and Evening (21:00) time periods averaged from six test plant species. Significant differences between time periods were identified using post-hoc tests; time periods that do not share a letter show significant variation ($p < 0.05$), ns = no significance.

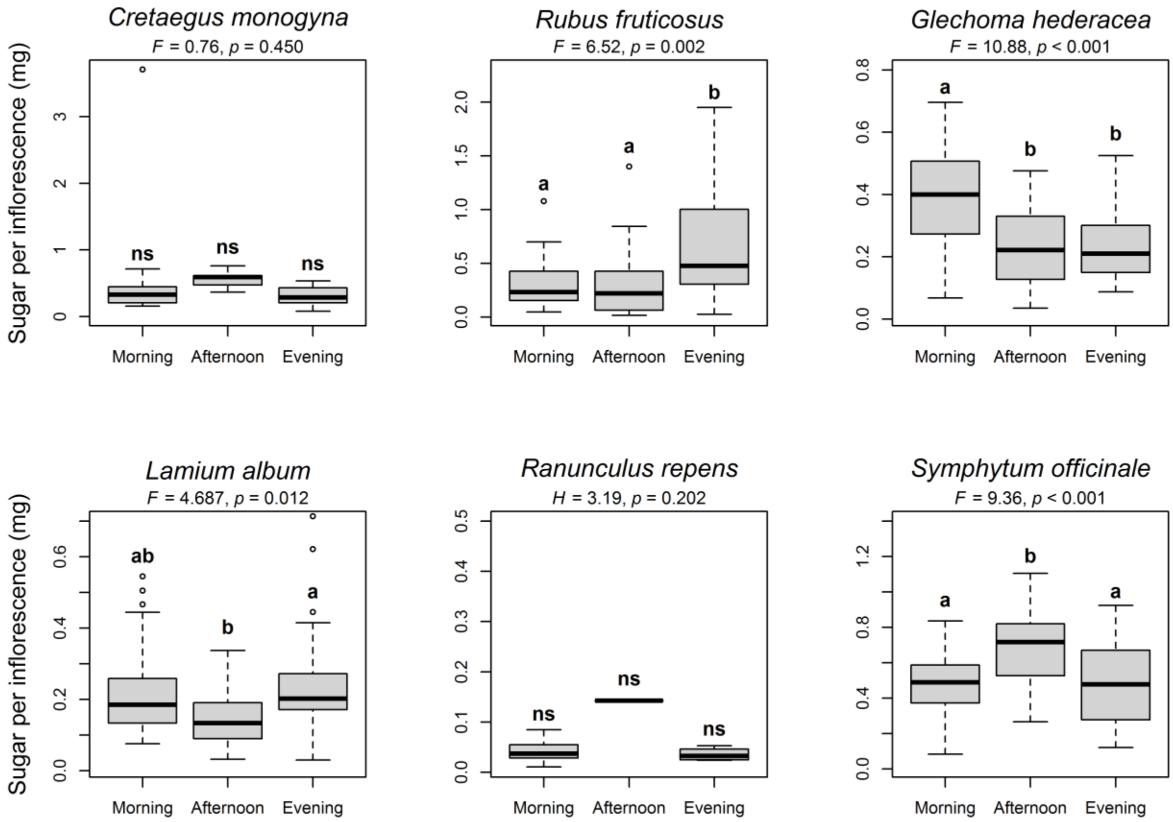

Fig. 3 Total sugar per inflorescence (mg) of test plant species at Morning (09:00 GMT), Afternoon (15:00) and Evening (21:00) time periods, with the test statistic ('F' for ANOVA and 'H' for Kruskall-Wallis, respectively) and significance levels for each analysis of variance between periods of the day for each species. Significant differences between time periods were identified using post-hoc tests; time periods that do not share a letter show significant variation ($p < 0.05$), ns = no significance.

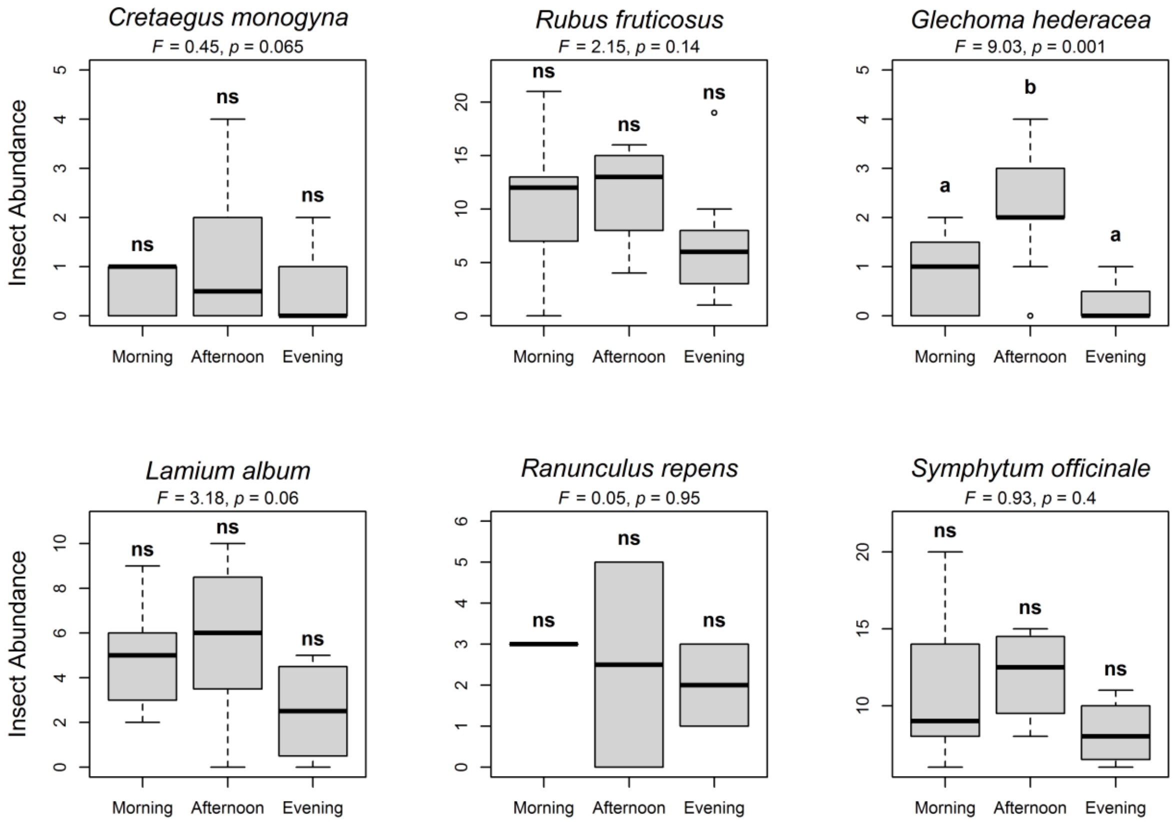

Fig. 4 Mean insect visits to test plant species in Morning (08:00—10:00 GMT), Afternoon (13:00—15:00) and Evening (19:00—21:00) time periods, with the test statistic and significance levels of ANOVAs between periods of the day for each species. Significant differences between time periods were identified using post-hoc tests; time periods that do not share a letter showed significant variation (p < 0.05).

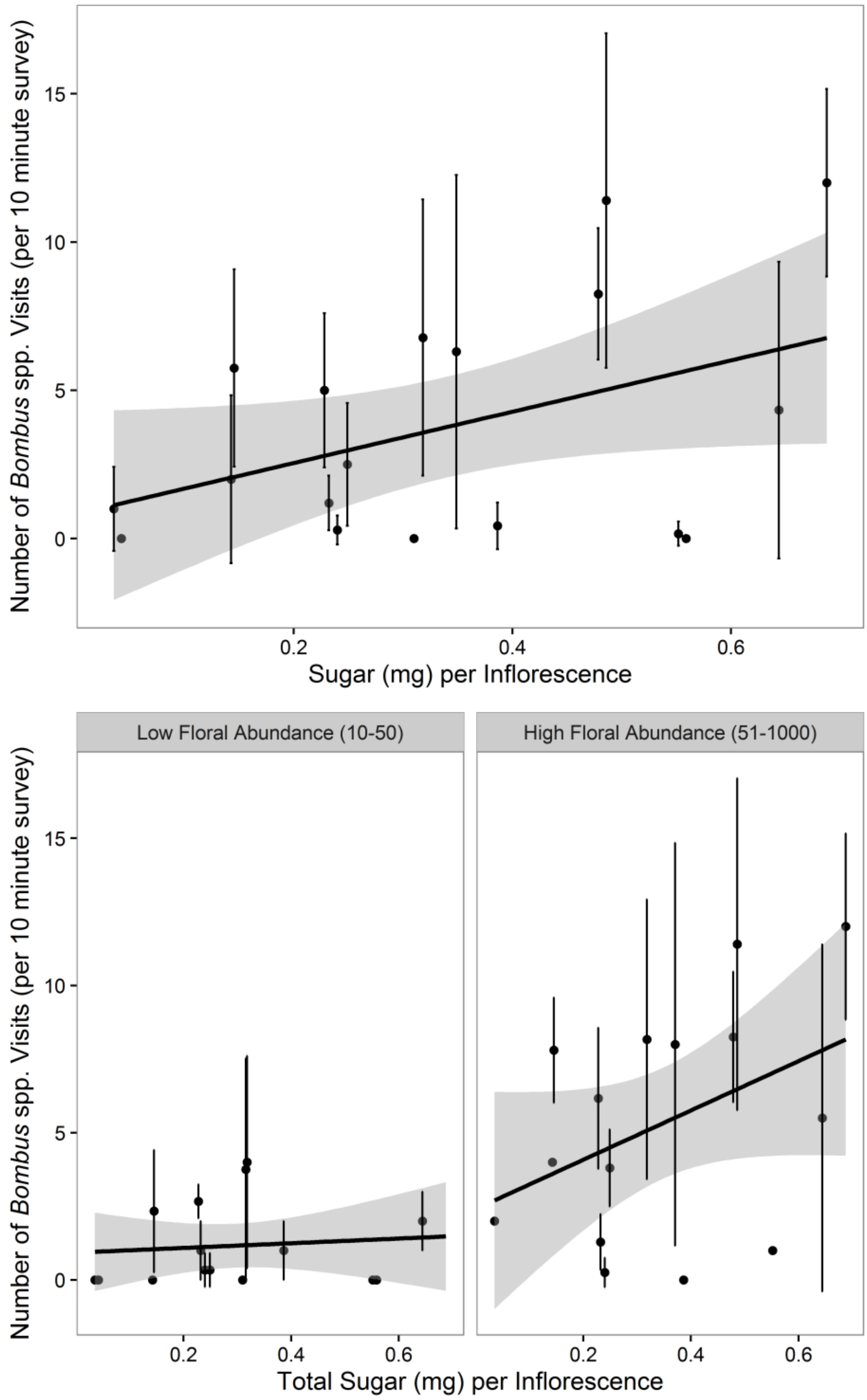

Fig. 5 Mean (± standard deviation) visits per test plant species to illustrate the positive effect of greater total sugar on visitation in *Bombus* spp. (above), and total sugar on *Bombus* spp. visits (below) when separated between sample plots with high or low floral abundance; low floral abundance (10—50 inflorescences per a 4 m^2, left) shows little or no trend, whilst high floral abundance (51—1000 inflorescences, right) shows a positive trend. Grey lines and polygons indicate model best fit and 95% Confidence Interval.